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BROWNING REACTION PRODUCTS OF CAKE CRUMB[1]

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ABSTRACT

The nature of the browning reaction products in cakes made with a high concentration of honey or reducing sugars has been investigated. Several of these browning-reaction products extracted from cake crumb have been separated by paper chromatography. One of these has an ultraviolet absorption maximum at 287–293 mμ. It rapidly reduces ammoniacal silver nitrate and 2,6-dichlorophenolindophenol in the cold and gives a deep blue color with aqueous ferric chloride. The ultraviolet spectrum and all other properties investigated show that the compound is not hydroxymethyl furfural or triose reductone. It is produced in the decomposition of 1-desoxy-1-piperidino-D-fructose and from the condensation products of glucose with phenylalanine and glycine. The compound browns rapidly with amino acids at a neutral pH and appears to be significant in the browning reaction.

Extensive studies on the browning or Maillard reaction (15) in recent years have dealt to a great extent with model systems of glucose and organic amines or amino acids. Browning with proteins is encountered in baking whenever reducing sugars, such as corn syrup, dextrose, invert, or honey, are used. Browning associated with these reducing sugars has been demonstrated to be of the Maillard type rather than caramelization. The phenomenon is accelerated in the presence of added amino acids (8).

From model systems, certain clear-cut reactions have been demonstrated. Crystalline N-glycosides of glucose with aromatic and aliphatic amines and their Amadori rearrangement products have been described. These reactions and their relationship to browning have been reviewed and restudied by Hodge (11, 12, 13). The Amadori rearrangement products upon heating undergo extensive degradation with the production of reductones, fluorescent compounds, and brown pigments (13). The amine base can be recovered in low yield, but glucose is not produced as it is from N-glycosides. It has been demonstrated by a large number of workers that glucose combines with amino acids. The noncrystalline products of Gottschalk (7) and Borsook (1) behave similarly to Amadori rearrangement products in that glucose cannot be recovered on hydrolysis. It is believed widely that browning with amino acids or proteins follows such an initial reaction. It has been

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demonstrated that glucose combines with the amino groups of protein in the solid state (14, 10). Irreversible changes soon follow. The brown pigments that are formed cannot be dialyzed or readily removed from the protein (16). These facts are in agreement with the studies on model systems.

From knowledge of model systems, certain generalizations about the two initial steps of the browning reaction can be made. The first step is reversible and is favored by neutral or alkaline conditions. In bakery products such as cake with a neutral pH and high sugar content, conditions are favorable for the first step of the reaction. The Amadori rearrangement is, however, acid-catalyzed. As in the case of amino acids (1), the catalysis with protein could be provided by the carboxyl group.

In the decomposition of Amadori rearrangement products some fragmentation occurs with the liberation of nitrogen-free components. This report treats of the isolation of such sugar fragments from cake and the comparison of them with fragments obtained from the Amadori rearrangement products of the reaction of glucose with phenylalanine, glycine, and piperidine. Evidence is presented that the Amadori rearrangement may take place in the browning of glucose with protein during baking.

Materials and Methods

Cake Extract. A white pound-cake formula was used. Sucrose was replaced by glucose so as to cause browning, and the shortening was omitted to simplify the isolation procedure. A typical crumb pH was 6.8 to 7.3. The brown crust was cut away and only the center crumb, which had browned very little, was placed in a beaker and extracted several times with ethyl acetate. The ethyl acetate extract was concentrated in vacuum to a small volume. The concentrated extract was applied directly to filter paper and chromatographed in water-saturated n-butanol containing 1–2% acetic acid. Cakes prepared with sucrose were treated in like manner for comparison.

Browned Casein Extract. A mixture of 100 g. purified casein and 100 g. glucose was mixed with sufficient water to form a stiff dough. The dough ball was placed in an oven at 100°C. for 1½ hours. Browning throughout the dough ball was evident. The mixture was finely pulverized and extracted with ethyl acetate as described above for cake crumb.

Caramel Extract. An ethyl acetate extract was obtained from a glucose caramel prepared by heating a glucose syrup until a pronounced color was evident.

Amadori Rearrangement Products of Model Systems. 1-Desoxy-1-piperidino-D-fructose (DPF) was prepared according to the procedure of Hodge (13). Fructose-glycine was prepared according to the procedure of Borsook (1). Fructose-phenylalanine was prepared by the method of Gottschalk (7). This product was not completely homogeneous, as has also been reported by Gottschalk (7).

The degradation products of these preparations, on heating in aqueous solution, were examined by paper chromatography and compared with cake and protein extracts.

Hydroxymethyl Furfural. Hydroxymethyl furfural was prepared by the method of Haworth and Jones (9).

Triose Reductone. Triose reductone was prepared by the method of Castelfranchi (2), a modification of the procedure of Euler and Martius (5, 6).

Paper Chromatography. Whatman No. 1 filter paper was used. For examination of the decomposition products of the Amadori rearrangement products, a developing solvent of water-saturated n-butanol containing 1% acetic acid was used. For detection of amino acids, the chromatograms were sprayed with a 0.1% solution of ninhydrin in n-butanol. Reducing compounds were detected by spraying with ammoniacal silver nitrate and 2,6-dichlorophenolindophenol solution.

Results and Discussion

A 5% solution of DPF, adjusted to pH 6.8 with acetic acid, was heated at 90°C. and samples were withdrawn at various time intervals and chromatographed (Fig. 1). Cake crumb, caramel, and browned protein extracts are compared with a heated DPF solution in Fig. 2. The compound marked 3 was of interest because it appeared to be present in all the samples. Compound 3 possessed strong reducing properties, since it reduced ammoniacal silver nitrate in the cold, requiring from 1 to 5 minutes for the spot to develop. However, with the caramel extract the resolution was not good. Hence, the presence of compound 3 in the caramel extract could not be established. There were a number of fluorescent compounds in the protein extracts shown as open spots, not present in the heated DPF. It is difficult to evaluate the significance of these. However, the formation of compound 3 in fair yield from DPF and its apparent presence in browned protein extracts appeared to be significant. Accordingly, the fructose-phenylalanine and fructose-glycine preparations were heated in the same manner. In both cases, as well as with DPF, the formation of compound 3 occurred before visual browning was apparent. These compounds also yielded the elongated spot No. 4. This is apparently a mix-

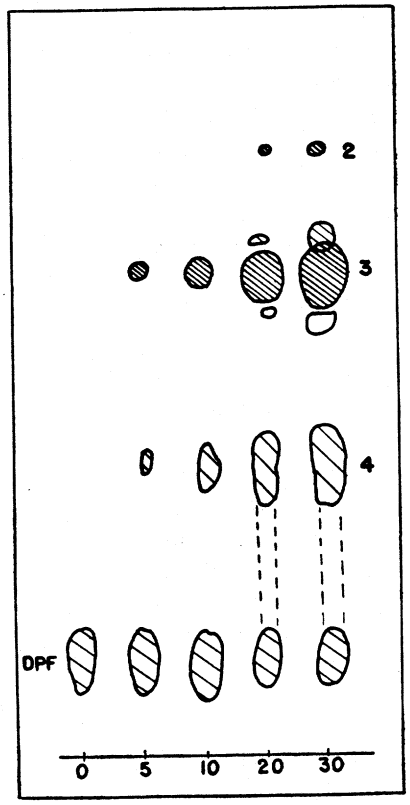

Fig. 1. The decomposition of 1-desoxy-1-piperidino-D-fructose (DPF). Abscissa, time of heating in minutes. 2 and 3 reacted rapidly with the silver nitrate spray. Dotted lines indicate a fluorescent streak.

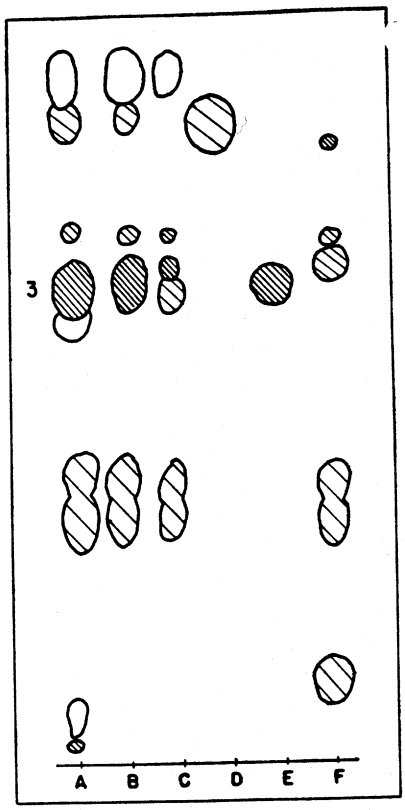

Fig. 2. Compound 3 from various sources. A, cake extract; B, glucose-casein extract; C, caramel extract; D, hydroxymethyl furfural standard; E, triose reductone standard; F, heated DPF solution.

ture of compounds that oxidize with difficulty. The presence of hydroxymethyl furfural (Fig. 2) in cake extracts was verified by spraying chromatograms with resorcinol in hydrochloric acid (7).

It also has been noticed that the pH of the solution is critical. If a solution of DPF is heated at pH 10, browning is extremely rapid. There is produced, in addition to compound 3, a true reductone, shown in Fig. 1 as spot No. 2. At pH 10, this compound was produced in higher yield than compound 3, whereas at pH 6.8 (Fig. 2) this reductone was present only in traces after 30 minutes of heating.

Compound 3 from the cake extract was obtained in homogeneous form by eluting the appropriate sections of several chromatograms with water and lyophilizing the extract. The compound was obtained as a dark oil (yield 40 mg. from 500 g. cake crumb). When rechromato-

graphed, traces of impurities were found. All attempts to crystallize this material failed.

Despite the low yield and noncrystalline character, certain definite information was obtained regarding the chemical properties of compound 3.

A water solution of the compound is weakly acidic. It could, however, be titrated to a definite phenolphthalein end point if the standard sodium hydroxide was added slowly with constant agitation over a period of 10 to 15 minutes. A definite stable end point was reached finally, corresponding to a neutralization equivalent of 190. Because of the syrupy nature of the product, this is undoubtedly high. The manner of the neutralization, however, indicates that if a carboxyl group is being titrated it must be bound, possibly as a lactone ring. However, it is more likely that enolic hydrogen is being titrated.

A nitrogen analysis was made by the micro diffusion method (3) and was negative.

The compound yields a deep-blue color with ferric chloride indicating enolic hydrogen. In this respect it is like triose reductone (4), produced by strong alkaline degradation of glucose. Note also the R_f of the two compounds are almost identical (Fig. 2). Compound 3 could be distinguished from triose reductone, however, by the fact that it gave no color reaction with aniline.

The compound is not so readily oxidized as triose reductone but it reduced 2,6-dichlorophenolindophenol. Acidic silver nitrate oxidized compound 3 very slowly. Fearon's *o*-dinitrobenzene test for enediols was negative.

The compound absorbs very strongly in the ultraviolet range of 287 to 293 mμ (Fig. 3). The spectrum is different from that of hydroxymethyl furfural, a compound frequently associated with browning. The R_f value in water-saturated n-butanol (Fig. 2) is also distinctly different from that of hydroxymethyl furfural.

The samples of compound 3 used to obtain these spectra (Fig. 3) were eluted from paper chromatograms and diluted with water so that all of them had approximately the same optical density at 290 mμ. The positions of the maxima in all the samples are almost identical. They differ mainly in the height of the peak which occurs below 230 mμ. The relative height of this peak, however, varies with the extent of browning and apparently is an impurity. This is illustrated by curves B and E (Fig. 3) obtained from two different samples of compound 3 from DPF. They differ only in that DPF had been heated for 30 minutes in the case of curve B and 10 minutes for curve E. The impurity has shifted the position of the maximum slightly.

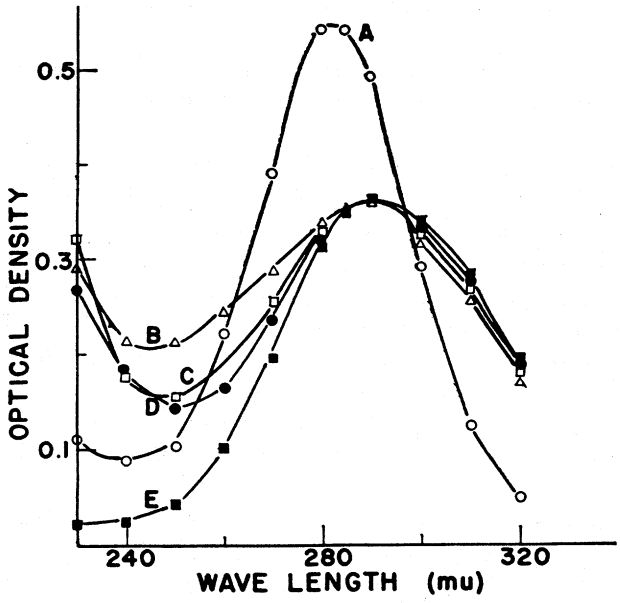

Fig. 3. Ultraviolet spectra of compound 3 as compared with hydroxymethyl furfural. A, hydroxymethyl furfural standard; B, compound 3 from DPF which was heated 30 minutes; C, compound 3 from glucose-casein extract; D, compound 3 from cake extract; E, compound 3 from DPF which was heated for 10 minutes.

The compound yielded a precipitate with 2,4-dinitrophenylhydrazine that could not be recrystallized to a constant melting point. Similarly, with benzoylchloride, a noncrystalline derivative was obtained. The latter was readily oxidized with aqueous potassium permanganate

The compound is rapidly destroyed on heating at 100°C. in dilute alkali or with glycine. With amino acids extremely rapid browning took place at pH 7.0. Ten to 20 minutes of heating in the presence of glycine at pH 7.0 resulted in almost complete destruction of the compound. Paper chromatography revealed that a number of compounds were formed, some of which fluoresced.

Chichester, Stadtman, and Mackinney (3) in 1952 separated 24 components from a heated glucose-glycine mixture. Their chromatographic techniques were similar to those reported in this work. Compound 3 may be identical with compound 7 described by them.

Interest was attached to the compound referred to as compound 3 because it appeared to be produced through caramelization as well as through the Amadori rearrangement product. It was demonstrated that traces of amino acids catalyze its formation in glucose syrup with heating. Two tubes of 80% glucose syrup were heated at 100° C. To one tube was added a trace of glycine (molar ratio of glucose to glycine

100:1). One-milliliter samples were withdrawn at various time intervals and extracted with 10 ml. of ethyl acetate concentrated to dryness and dissolved in 0.5 ml. water. Examination by paper chromatography revealed that compound 3 was produced in the tube containing the amino acid after 15 minutes of heating, whereas in the glucose syrup it was not evident even after 2 hours of heating. From the work with synthetic Amadori rearrangement products, it appears that the catalysis is through this rearrangement and that the rearrangement takes place in glucose-protein systems such as occur in bakery products.

It was observed by Hodge (13) that DPF on heating yields free piperidine. It has been noted, chromatographically, that the formation of compound 3 occurs simultaneously with the formation of the free base. This is also true with the amino-acid condensation products. The free amino acid can be detected by spraying a chromatogram with ninhydrin. It was of interest to obtain quantitative data on the formation of compound 3. A 5-g. sample of DPF was dissolved in water and adjusted to pH 6.8 with acetic acid and made up to 50 ml. The solution was then heated at 85°C. Samples were withdrawn and spotted for chromatographic separation along with a standard containing compound 3. The remaining samples were diluted to 5 ml. with water and the optical densities measured at 500 mμ as a measure of color development. The chromatograms, after development, were sectioned and the portion containing the standard was sprayed with silver nitrate solution. Compound 3, the location thus revealed, was eluted with water and the optical density measured at 287 mμ. The data are presented in Table I.

TABLE I

MEASUREMENT OF THE DECOMPOSITION PRODUCTS OF DPF[a]

TIME	OPTICAL DENSITY OF ENTIRE SAMPLE (500 mμ)	OPTICAL DENSITY OF COMPOUND 3 OBTAINED FROM 6.7-μl. SAMPLE (287 mμ)
minutes		
0	0.0	0.0
3	0.001	0.0
6	.004	0.0
9	.003	0.01
12	.011	.035
15	.017	.055
18	.038	.087
21	.068	.115
24	.106	.155
27	.182	.167
30	.264	.190
33	.352	.230
36	.512	.270
39	0.650	0.275

[a] 1-Desoxy-1-piperidino-D-fructose.

It is evident that significant amounts of compound 3 are formed near the beginning of the heating and the amount increases gradually. Browning, however, is accelerated as heating is continued. This is shown graphically in Fig. 4.

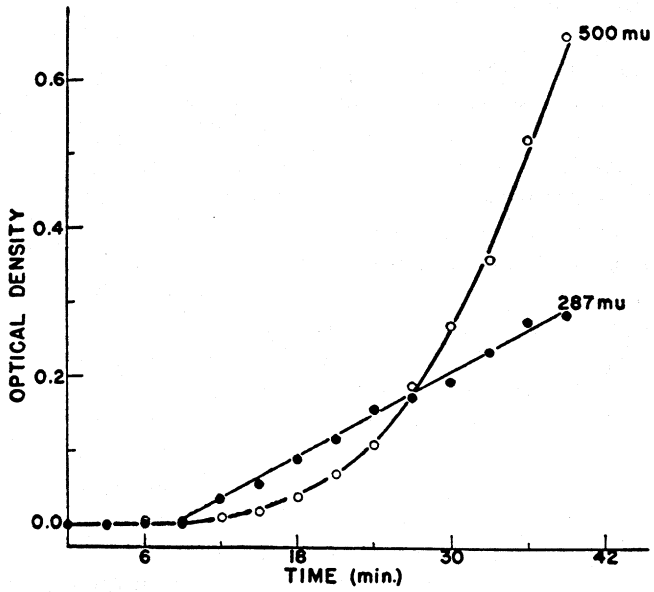

Fig. 4. Development of compound 3 during browning of DPF. Browning was measured at 500 mμ.

In another experiment various volumes of heated DPF were applied to paper, and after development the appropriate sections were eluted. Measurement at 287 mμ yielded the results reported in Table II.

When the results are plotted on a graph, an approximately linear relationship is found between optical density and volume of solution. The total errors of the method, largely pipetting, varied from 5 to 20%. The optical density readings at 287 mμ reported in Table I and

TABLE II

OPTICAL DENSITY OF COMPOUND 3 AS A FUNCTION OF CONCENTRATION

VOLUME OF HEATED DPF[a]	OPTICAL DENSITY OF ELUTED COMPOUND 3 (287 mμ)
μl.	
0.0	0.0
6.7	0.101
13.4	.195
20.1	.350
26.8	.460
33.5	.600
40.2	0.730

[a] 1-Desoxy-1-piperidino-D-fructose.

Fig. 4 are thus proportional to the concentration of compound 3 in the sample.

Conclusions

The work reported here is evidence that, under bakery conditions, sugar amine condensation proceeds as postulated by many workers. An important stage after the Amadori rearrangement, however, is one where the nitrogen body is cleaved from the sugar fragment. Browning must result to a considerable extent when the highly reactive sugar fragment recombines in some way with the nitrogen-containing species. The composition of the sugar fragment referred to as compound 3 remains unknown. Characterization of this compound should prove interesting.

Acknowledgment

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